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Bone health of adult hemodialysis patients is related to vitamin K status

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Bone health of adult hemodialysis patients is related to vitamin K status. This investigation of 68 hemodialysis patients (ages 33 to 91) analyzed the association of biochemical indicators of vitamin K nutriture and bone metabolism, and related both to past bone fracture history and prospective bone fracture risk. Phylloquinone concentrations were significantly lower in the 23 patients with previous fractures compared to those without (0.93 vs. 1.50 nmol/liter, $P < 0.003$) and a smaller percentage of their serum osteocalcin was carboxylated (48.8 vs. 53.6%, $P < 0.03$). The 41 patients who never had fractures had nearly three times higher phylloquinone concentrations than the nine patients with fractures during a four-year follow-up period (1.59 vs. 0.55 nmol/liter, $P < 0.002$) and more carboxylated serum osteocalcin (55.2 vs. 42.0%, $P < 0.01$). None of the patients with phylloquinone concentrations over 2.2 nmol/liter had elevated intact parathyroid hormone (iPTH) concentrations, and only patients with less than 1 nmol/liter phylloquinone had severe hyperparathyroidism (iPTH>300 ng/liter). Our data thus indicate that suboptimal vitamin K nutriture in hemodialysis patients is associated both with increased bone fracture risk and with a high prevalence of hyperparathyroidism.

Vitamin K is an essential cofactor for the site-specific carboxylation of osteocalcin and other bone matrix proteins [1]. Biochemical indicators of reduced vitamin K status in otherwise healthy adults are associated with increased urinary calcium losses [2], reduced mineral density of the hip bone [3] and increased risk of bone fracture [4]. Those who have already suffered a bone fracture have been found to have lower vitamin K concentrations in serum than age-matched controls [5–7]. Long-term use of vitamin K supplements normalized urinary calcium losses [8] and maintained bone mineral density [9]. A year-long placebo-controlled trial in hemodialysis patients showed a similarly favorable effect of vitamin K supplements on bone density [10].

Less than optimal vitamin K status in otherwise healthy populations may be much more common than previously thought, particularly in the elderly [2, 11, 12]. A recent investigation [13] indicated that vitamin K status of middle-aged and elderly hemodialysis patients is similarly precarious; one out of four hemodialysis patients had a serum phylloquinone concentration that was at least two SDs below the mean of healthy adults. The relevance of these findings was not clear, particularly in light of an earlier report of higher than normal phylloquinone concentrations in a majority of hemodialysis patients [14]. The aim of the present study was, therefore, to determine how differences in bone health of hemodialysis patients might be related to differences in vitamin K nutriture. To this end we compared vitamin K status with the history and four-year prospective risk of bone fractures.

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Patients

Hemodialysis patients were recruited from three hemodialysis centers in Berlin, Germany. All patients were clinically stable and without acute infectious disease. They had been undergoing hemodialysis on an outpatient basis for at least one month. No patients were receiving vitamin K-antagonists at the time of blood sampling.

All patients received 50,000 IU of vitamin D at the begin of every quarter, each time in five oral doses spread over five days. Additional calcitriol therapy was rarely used. Patients were routinely dialyzed against solutions containing 1.75 mmol/liter calcium.

Informed consent was obtained from all patients prior to the study. The investigation was approved by the Institutional Review Board of the Berlin Medical Association, Berlin, Germany.

Analytical methods

Bone fracture history. Information about previous fractures was compiled from all available patient records both before and after their diagnosis of renal disease. All patients had undergone annual chest X-ray examinations from the start of hemodialysis treatment. All recorded references to a bone fracture were counted, whether these were obtained from dated hospital records or were part of the patient's medical history, regardless of cause, severity, location or age.

Blood samples. All patients observed an overnight fast before blood for vitamin K and other analyses was obtained. The preceding hemodialysis session always had been completed at least thirty hours earlier. On the morning of the day of the study, blood was drawn from an antecubital vein into plain and EDTA-coated syringes for the preparation (within 2 hr) of serum or plasma, respectively. All samples were protected from light during collection, storage (−20°C or lower) and analysis.

Biochemical analyses. Phylloquinone was analyzed by a multistage procedure [15]. The consecutive steps were extraction of

Table 1. Indicators of vitamin K nutriture and bone metabolism in hemodialysis patients with a history of bone fractures at the time of recruitment compared to those who did not have fractures until then

	Fractures	(*N*)	No fractures	(*N*)	*P*
Age *years*	67.3 ± 2.5	(23)	62.3 ± 1.8	(45)	< 0.15
Years on hemodialysis	5.5 ± 0.9	(23)	3.5 ± 0.4	(45)	< 0.08
Phylloquinone *nmol/liter*	0.93 ± 0.25	(23)	1.50 ± 0.20	(45)	< 0.003
Hydroxylapatite-binding osteocalcin %	48.8 ± 2.2	(19)	53.6 ± 1.6	(39)	< 0.03
Total osteocalcin	26.0 ± 6.4	(19)	23.8 ± 3.6	(39)	NS
Intact parathyroid hormone *ng/liter*	228 ± 64	(20)	165 ± 50	(36)	NS

Data are means and standard errors of the mean (SEM). The number of patients for which data are available is given in parentheses (NS indicates $P < 0.25$).

Table 2. Indicators of vitamin K nutriture and bone metabolism in hemodialysis patients who suffered bone fractures after recruitment compared to those who never had fractures

	Fractures	(*N*)	No fractures	(*N*)	*P*
Age *years*	66.0 ± 4.5	(9)	61.7 ± 1.8	(41)	NS
Years of hemodialysis	6.7 ± 0.8	(9)	3.4 ± 0.5	(41)	< 0.14
Phylloquinone *nmol/liter*	0.55 ± 0.08	(9)	1.59 ± 0.21	(41)	< 0.002
Hydroxylapatite-binding osteocalcin %	42.0 ± 2.9	(8)	55.2 ± 1.6	(35)	< 0.01
Total osteocalcin	34.0 ± 6.7	(8)	23.4 ± 4.0	(35)	< 0.15
Intact parathyroid hormone *ng/liter*	180 ± 66	(7)	160 ± 47	(36)	NS

Data are means and standard errors of the mean. The number of patients for which data are available is given in parentheses. Differences between the groups were tested by Student's *t*-test (NS indicates $P > 0.25$).

lipophils from serum, purification by sorbent extraction and normal-phase high-performance liquid chromatography (HPLC), final separation by reversed-phase HPLC, and measurement of the vitamin by dual-electrode electrochemical detection in the redox-mode. Menaquinone-6 was used as an internal standard to correct for losses. All measurements were carried out at least in duplicate and the means of the results were used for data analysis. Interassay variation (variation coefficient) was 11%. Undercarboxylated osteocalcin was separated from the carboxylated form by incubating serum with hydroxylapatite [16]. Osteocalcin was measured in untreated serum and in hydroxylapatite-treated serum by radioimmunoassay (OSCAtest Osteocalcin, Henning, Berlin, Germany). Interassay variation for the measurement of the undercarboxylated osteocalcin fraction was 16%. The concentration of intact parathyroid hormone (iPTH) was measured with a non-radioactive immunoassay (LIAmat iPTH, Byk-Sangtec, Dietzenbach, Germany) with an interassay variation of 8%.

Statistics

Group differences were tested with the Kolmogorov-Smirnov two-sample test for continuous variables and with Chi-square statistics for fracture history. All calculations were carried out with a statistical software package (Statgraphics 2.4, SCSC) on a personal computer.

Results

Bone fracture history

A review of all available X-ray records and patient charts showed that 23 of the 68 patients had suffered one or more documented fractures prior to recruitment. In most cases (16 of 23) some or all of the fractures had occurred before hemodialysis had become necessary. During the four years following recruitment nine patients suffered new fractures; five of these had had fractures previously.

Indicators of vitamin K nutriture

Serum phylloquinone concentrations varied widely in these patients, ranging from 0.14 to 5.0 nmol/liter with a mean of 1.33 nmol/liter. Concentrations were not significantly related to gender or age. Mean serum phylloquinone concentration (Table 1) was 38% lower in patients with a history of bone fractures at the time of recruitment than in those without (0.93 vs. 1.50 nmol/liter, $P < 0.003$). There was an almost threefold difference (Table 2) in phylloquinone concentrations of the nine patients who had bone fractures during four years following recruitment and the 41 patients who never had a fracture (0.55 vs. 1.59 nmol/liter, $P < 0.002$).

The proportion of serum osteocalcin that binds to hydroxylapatite *in vitro*, as a measure of the completeness of vitamin K-dependent γ-carboxylation of osteocalcin in bone [16], was assayed in 58 of the patients. Measurements were not carried out for the other 10 patients, because serum samples had been collected inappropriately or not at all. The proportion of hydroxylapatite-binding osteocalcin (HBC) was 9% lower in patients with fractures prior to recruitment than in those who had not had a fracture until then (48.8 vs. 53.6%, $P < 0.03$). HBC also was significantly lower in the patients who later had fractures compared to those who never had fractures. No statistically significant effects of fracture history or prospective bone fracture risk on serum osteocalcin concentrations were observed.

The percentage of hydroxylapatite-binding osteocalcin was correlated with phylloquinone concentration ($r = 0.28, P < 0.05$). No statistically significant relationships were found between serum phylloquinone concentrations and the concentrations of hydroxylapatite-binding or non-binding osteocalcin.

Indicators of bone metabolism

Total osteocalcin concentrations in serum, an indicator of osteoblast activity, did not differ significantly between patients

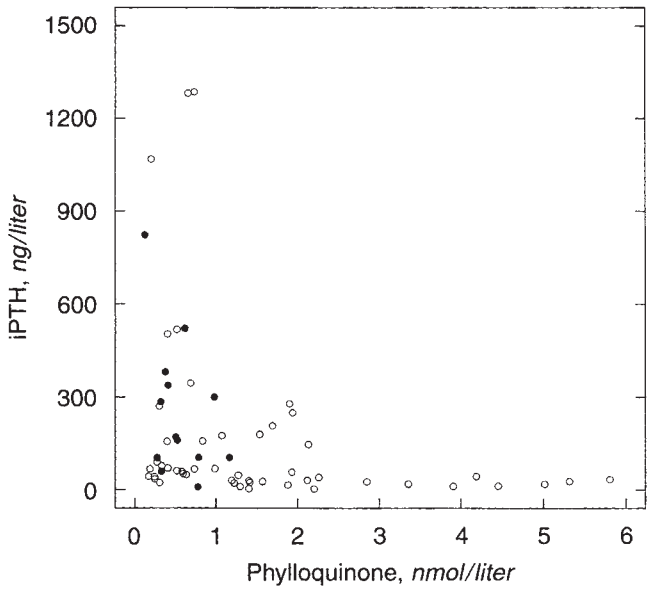

Fig. 1. *Relationship between serum concentrations of phylloquinone (vitamin K_1) and intact parathyroid hormone in 63 of the hemodialysis patients.* Closed circles indicate patients who had fractures after starting hemodialysis treatment.

with and without previous bone fractures. All patients had concentrations above the normal reference limits with values ranging from 2.5 to 120 μg/liter. Thirty-one of the 63 patients for whom results were available had elevated plasma concentrations (> 60 ng/liter) of intact parathyroid hormone (iPTH), which is known to increase serum osteocalcin concentrations [17]. iPTH concentrations tended to be higher in patients who had had previous fractures than in those who had never had fractures, but the difference was not statistically significant (Table 1). The same pattern and absence of statistical significance of the difference was observed in respect to prospective bone fracture risk (Table 2).

Plasma iPTH concentrations were related to serum phylloquinone concentrations (Fig. 1). A statistically significant correlation was obtained after logarithmic transformation of both parameters ($r = -0.45$, $P < 0.01$). None of the ten patients with serum phylloquinone concentrations over 2.2 nmol/liter had an elevated plasma iPTH, but 45% of patients with phylloquinone concentrations between 1.0 and 2.2 nmol/liter had elevated iPTH concentrations, and 71% of patients with phylloquinone concentrations below 1 nmol/liter. Only patients with a serum phylloquinone concentration of less than 1 nmol/liter had iPTH concentrations in excess of 300 ng/liter. Among the patients for whom iPTH values were available 13 had fractures after starting hemodialysis; all but one of these presented evidence of both suboptimal vitamin K status and hyperparathyroidism ($P < 0.02$). Vitamin K-replete patients, in contrast, appeared to have escaped both hyperparathyroidism and bone fracture during hemodialysis treatment.

Discussion

Chronic renal failure causes severe disturbances of bone mineral metabolism that are not completely corrected by hemodialysis treatment. Phosphate retention and impaired synthesis of 1,25-dihydroxyvitamin D are common problems that are thought to excessively stimulate parathyroid hormone secretion and which may cause the accelerated loss of bone minerals. This study was undertaken to determine whether poor vitamin K status might contribute to the pathogenesis of renal osteodystrophy. From this investigation two major findings emerged: Vitamin K status was poorer in patients with fractures than in those without; and hyperparathyroidism was most severe in patients with poor vitamin K status.

The mechanism by which vitamin K might protect against bone fracture is not known. While it is known that vitamin K is needed for the synthesis of mature osteocalcin, matrix Gla protein and protein S in bone, the specific function of these vitamin K-dependent proteins is still obscure. The finding that only patients with low phylloquinone concentrations had severely elevated iPTH concentrations was unexpected. The close relationship between vitamin K and iPTH concentrations may suggest that a vitamin K-dependent process is important for the regulation of parathyroid hormone. This could involve a calcium-binding protein that is both vitamin K and vitamin D dependent, as is the case with osteocalcin in bone.

Serum phylloquinone concentrations have previously been found to be strongly dependent on apolipoprotein E genotype [13, 18]. Since this genetic trait is constant throughout life, current phylloquinone concentrations may be indicative of past concentrations. This would explain how a slowly evolving event like bone fracture can be linked to a parameter as volatile as vitamin K concentration in serum.

The preventive and therapeutic potential of maintaining optimal vitamin K status may be considerable. Patients with serum phylloquinone concentrations of 1.2 nmol/liter or less had suffered bone fractures at a rate of more than 6%/year during hemodialysis treatment whereas none of the others had fractures. More than half of the hemodialysis patients at our centers (and probably elsewhere) had serum phylloquinone concentrations below this threshold and might benefit from supplemental vitamin K. Preliminary results from a placebo-controlled 12-month intervention trial in hemodialysis patients [10] indicate that vitamin K supplementation helps to conserve bone mineral mass and may thereby decrease bone fracture risk. Apart from its effect on bone, we are currently investigating whether vitamin K can decrease elevated iPTH concentrations and thereby alleviate one of the most obstinate problems in the treatment of end-stage renal failure.

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